Adenovirus Generation. High titer adenovirus expressing c-MYC or MadMyc was generated using the AdEasy system as described (8). In brief, a fragment containing the CMV-promoter and a human c-*MYC* cDNA fused to an HA-epitope-tag was excised from the construct HH67 (9) using the restriction enzymes Xho I and Hind III and inserted into the shuttle vector pAdTrack. To generate an HA-epitope tagged MadMyc cDNA, the previously described MadMyc encoding plasmid (10) was employed as a template in a PCR using the primers A3 5'-GTCTCAGGTACCTTCCACCATGGCGGCGGCGGTTCGG-3' (SEQ ID NO: 1) and 5'-GATCATCGATGTTATTGTATGGTAACATGG-3'(SEQ ID NO: 2). The resulting fragment was cut with *Kpn* I and *Cla* I and ligated into the HH67 vector (see above) digested with the same enzymes. A fragment containing the CMV-promoter and the MadMyc-ORF was then transferred to pAdTrack. After recombination with the vector pAdEasy, high titer virus was generated in 911 and 293 cells. Viruses were purified via a CsCl gradient and the effective titer was determined by the frequency of GFP positive cells after infection. The efficiency of the infection was normalized to the frequency and intensity of GFP positive cells.

At page 13, paragraph 1, substitute the following paragraph:

Isolation of the Human and Murine *CDK4* Genes. The primer pair 5'-CAGCATCACCTCTGGTACCC-3' (SEQ ID NO: 3) and 5'-CCCGAATTCCGGGGCGAACGCCGGACG-3' (SEQ ID NO: 4) respectively, derived from the cosmid sequence ((13) and GenBank HSU81031) containing the *CDK4* promoter region was used to screen a human BAC library. A BAC (662M22, Research Genetics) containing the *CDK4* promoter was digested with *Kpn* I. A 2 kb A4 fragment containing the *CDK4* promoter was identified using PCR and then subcloned into pBR322 (corrected sequence deposited as GenBANK entry AF224272). For isolation of the murine *cdk4* gene the primer pair 5'-CTGCCACTCGATATGAACCCG-3' (SEQ ID NO: 5) and 5'-TAGATCCTTAATGGTCTCAACCG -3' (SEQ ID NO: 6) derived from the mouse *Cdk4* cDNA was used to identify a BAC (509, Research Genetics) containing the mouse *Cdk4* gene. A 4 kbp *Kpn* I fragment containing the promoter, exon 1 and 2 and the first intron was then subcloned into pBR322 and partially sequenced (sequence deposited as GenBANK AF223390).

At page 15, paragraph 1, which spans page 16, substitute the following paragraph:

Reporter Assays. To generate reporter constructs, the following oligonucleotides were used:

5'-CCGGTACCGGGTTGTGGCAGCCAGTCACGTGCCCGCCGCGTAGCCACACCTCTGCTCCTCAGAGCAATGT CAAGCGGTCACGTGTGATAGCAACAGATCACGTGGCTGCCATCGCCCCTC-3' (Oligo A, for wild type MBS 1-3; SEQ ID NO: 7), 5'-ATGAATTCCGGACGTTCTGGGCACGTGACCGCCACCCATG CGCTGAGGGGCGGACAGGAGGTGCTTCGACTGGGAGGAGGGCGAAGAGTGTAAGGGGGCGGAGGGGCGAT GGCAGCC-3' (Oligo B, for wild type MBS 4; SEQ ID NO: 8), 5'-CCGGTACCGGGTTGTGGCAGCCAGTCACCTGCCCGCCGCGTAGCC

ACACCTCTGCTCCTCAGAGCAATGTCAAGCGGTCACCTGTGATAGCAACAGATCACCTGGCTGCCA

TCGCCCCTC -3' (Oligo C, for mutant MBS 1-3; SEQ ID NO: 9), and 5'-ATGAATTCCGGACGTTCTGGGCAGGTGACCGCCACCCATGCGCTGAGGGGCGGACAGGAGGTGCTTCGA CTGGGAGGAGGGCGAAGAGTGTAAGGGGGCGGAGGGGCGATGGCAGCCAGG-3' (Oligo D, for mutant MBS 4; SEQ ID NO: 10). Different combinations of oligonucleotide pairs (A+B, A+D, C+B, C+D) were annealed and converted to double stranded fragments through 1 PCR cycle. These promotor fragments were subcloned into the *Kpn I/BamH* I sites of pBV-luc, a modified pGL3-basic derived reporter containing a minimal promoter (15). Further polymerase-derived mutants (mutMBS2 and mutMBS3+4) were identified while sequencing the reporter constructs. For reporter assays in RAT1 cells, transfections were performed using Lipofectamine (Life Sciences), 1 mg of reporter plasmid and 0.1 mg of a β-galactosidase reporter to control for transfection efficiency. Luciferase and β-galactosidase activities were assessed 24 h following transfection-using reagents from Promega and ICN Pharmaceuticals, respectively. To test the

ability of exogenous cMyc to transactivate reporters, subconfluent NIH3T3 fibroblasts were transfected by Lipofectin (Gibco) with 2 mg of reporter plasmid and different amounts of either MLV-LTR driven plasmids expressing wild type c-Myc or mutant c-Myc with the helix-loop-helix (HLH) domain deleted (deletion of amino acids 371-412) (16). Luciferase activity was measured 48 hours after transfection following the manufacturer's protocol (Promega). Total DNA amount was equalized by adding different amounts of empty MLV-LTR vector.

A5

At page 17, paragraph 2, substitute the following paragraph:

Retrovirus Generation. The *CDK4* ORF was generated by PCR using the EST W77860 as a template and the primers 5'-GCGGATCCGCGGCCGCCTTCCACCATGGCTACCTCTCGATCTGAGC-3' (SEQ ID NO: 11) and 5'-CGGTCGACTCACTCCGGATTACCTTCATC-3' (SEQ ID NO: 12). The resulting product was digested with the enzymes *Not* I and *Sal* I and inserted into the respective sites of the vector G1BgSVNA (a retroviral vector encoding a hygromycin resistance gene and β-galactosidase) replacing the β-galactosidase gene. The unmodified vector was used as a control. Bosc23 packaging cells (7) were transfected and the supernatant of resistant, pooled cells was used to infect Rat1 cells.

A6

Respectfully submitted,

By: _____
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Dated: August 6, 2001

Appendix showing marked up version of the specification
(all paragraph references number paragraph 1 as the first paragraph *starting* on the indicated page)

At page 4, paragraph 6:

Fig. 2 B. Alignment of the human (SEQ ID NO: 13) and mouse *CDK4* (SEQ ID NO: 14) promoter sequence upstream of the TSS. Identical residues are shaded black and the identical MBS are shaded gray.

At page 9, paragraph 5, which spans page 10:

Adenovirus Generation. High titer adenovirus expressing c-MYC or MadMyc was generated using the AdEasy system as described (8). In brief, a fragment containing the CMV-promoter and a human c-*MYC* cDNA fused to an HA-epitope-tag was excised from the construct HH67 (9) using the restriction enzymes Xho I and Hind III and inserted into the shuttle vector pAdTrack. To generate an HA-epitope tagged MadMyc cDNA, the previously described MadMyc encoding plasmid (10) was employed as a template in a PCR using the primers 5'-GTCTCAGGTACCTTCCACCATGGCGGCGGCGGTTCGG-3' (SEQ ID NO: 1) and 5'-GATCATCGATGTTATTGTATGGTAACATGG-3'(SEQ ID NO: 2). The resulting fragment was cut with *Kpn* I and *Cla* I and ligated into the HH67 vector (see above) digested with the same enzymes. A fragment containing the CMV-promoter and the MadMyc-ORF was then transferred to pAdTrack. After recombination with the vector pAdEasy, high titer virus was generated in 911 and 293 cells. Viruses were purified via a CsCl gradient and the effective titer was determined by the frequency of GFP positive cells after infection. The efficiency of the infection was normalized to the frequency and intensity of GFP positive cells.

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ACACCTCTGCTCCTCAGAGCAATGTCAAGCGGTCACCTGTGATAGCAACAGATCACCTGGCTGCCA TCGCCCCTC -3' (Oligo C, for mutant MBS 1-3; SEQ ID NO: 9), and 5'-ATGAATTCCGGACGTTCTGGGCAGGTGACCGCCACCCATGCGCTGAGGGGCGGACAGGAGGTGCTTCGA CTGGGAGGAGGGCGAAGAGTGTAAGGGGGCGGAGGGGCGATGGCAGCCAGG-3' (Oligo D, for mutant MBS 4; SEQ ID NO: 10). Different combinations of oligonucleotide pairs (A+B, A+D, C+B,

C+D) were annealed and converted to double stranded fragments through 1 PCR cycle. These promotor fragments were subcloned into the *Kpn I/BamH* I sites of pBV-luc, a modified pGL3-basic derived reporter containing a minimal promoter (15). Further polymerase-derived mutants (mutMBS2 and mutMBS3+4) were identified while sequencing the reporter constructs. For reporter assays in RAT1 cells, transfections were performed using Lipofectamine (Life Sciences), 1 mg of reporter plasmid and 0.1 mg of a β-galactosidase reporter to control for transfection efficiency. Luciferase and β-galactosidase activities were assessed 24 h following transfection-using reagents from Promega and ICN Pharmaceuticals, respectively. To test the ability of exogenous cMyc to transactivate reporters, subconfluent NIH3T3 fibroblasts were transfected by Lipofectin (Gibco) with 2 mg of reporter plasmid and different amounts of either MLV-LTR driven plasmids expressing wild type c-Myc or mutant c-Myc with the helix-loop-helix (HLH) domain deleted (deletion of amino acids 371-412) (16). Luciferase activity was measured 48 hours after transfection following the manufacturer's protocol (Promega). Total DNA amount was equalized by adding different amounts of empty MLV-LTR vector.

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